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16. ABSTRACT

Ozone alters root growth and root processes by first reducing photosynthesis and altering foliar metabolic pathways. The alteration in foliar metabolism is reflected in lowered carbohydrate levels in the roots. This can reduce key metabolic processes such as mineral uptake and symbiotic nitrogen fixation. These changes in root metabolism will ultimately be expressed in reduced plant growth and yield.

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JOURNAL ARTICLE

INTERACTION OF AIR POLLUTANTS AND MYCORRHIZAE

Patrick M. McCool and John A. Menge*

Mycorrhizal fungi are beneficial fungi which are associated with the roots of most plants. The mycorrhizal-host relationship is a symbiotic association in which the fungus receives energy in the form of fixed carbon (sugars) and, in return, provides the plant with an easy access to essential mineral nutrients which might exist in the soil environment in very low concentrations. Plants infected with mycorrhizae frequently show a growth increase over non-mycorrhizal plants. This growth increase may be due to: (1) increased efficiency of uptake of essential nutrients, (2) a decrease in the resistance to water transport in the roots, (3) production of plant growth hormones (auxins, cytokinins) and vitamins, or (4) increased resistance to attack by some plant pathogenic microorganisms. The gross morphology and structural characteristics of mycorrhizae allow them to be grouped into three classes: endomycorrhizae, ectomycorrhizae, and ectendomycorrhizae. Of these groups, only the endomycorrhizae and ectomycorrhizae will be considered here.

Figure 1. Effect of mycorrhizal infection and nutrient level on growth of citrus.

Ectomycorrhizae

Ectomycorrhizal associations are common on most forest trees. Ectomycorrhizal hosts are found primarily in the families Pinaceae, Betulaceae, and Fagaceae. Ectomycorrhizal symbionts are ascomycetes or basidiomycetes and most can be artificially cultured. Ectomycorrhizal roots differ greatly in morphology from non-mycorrhizal roots. Mycorrhizal roots are thicker and usually dichotomously branched. The entire root surface is covered by fungal hyphae forming a mantle or sheath and the hyphae extend into the intercellular spaces of the root cortical cells. The hyphae pack closely in these intercellular spaces to form the Hartig net which is important in nutrient exchange between the two symbionts. When intercellular penetration is not present, the association is considered asymbiotic.

Endomycorrhizae

The morphological and physiological characteristics of vesicular-arbuscular (VA) mycorrhizae, which are the most widespread type of endomycorrhizae, differ from those of ectomycorrhizae. VA mycorrhizae have a very wide host range and occur on the majority of crop plants in California and other parts of the world. It is easier to list the plant families in which VA mycorrhizae do not occur.

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This issue of CALIFORNIA AIR ENVIRONMENT is focused on the effect of air pollutants on plant systems, with emphasis on microflora and root processes, and on experimental design for mixed gas studies of plant responses to air pollutant exposure.

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INTERACTION . . .

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Most families except for the majority of plants in Cruciferaceae, Chenopodiaceae, Fumariaceae, Cyperaceae, Commelinaceae, Urticaceae, and Polygonaceae and endomycorrhizal. VA mycorrhizal fungi are obligate parasites and they must be grown on the roots of living hosts, such as sudan grass [*Sorgum sudanense* (Piper) Staph].

Endomycorrhizal infections produce little change in the external root morphology. They have a loose fungal network which extends a considerable distance into the soil. Intercellularly the fungi colonize epidermal and cortical cells but never invade the stele, endodermis, or root meristem. Infection usually occurs on root hairs or in the cortical cells behind the root meristematic region. Hyphae grow intra- and inter-cellularly in the host. Soon after infection, the fungi produce very complex dichotomously branched arbuscles within the cortical cells. Arbuscles are quickly digested and their contents are absorbed by the host. Presumably, this is one site for nutrient exchange in this symbiosis. Vesicles are also formed by the fungi and may serve as storage organs or as thick walled resting spores.

The endomycorrhizal symbiosis results in significant increases in growth and development (Figure 1). Inoculation of fumigated soil with endomycorrhizae has been shown to increase the growth of citrus by as much as 1600% (Kleinschmidt and Gerdemann, 1972), grape by 4900% (Bevingham and Obibnk, 1971), soybeans by 122% (Ross, 1971), and onions by 80% (LaRue et al., 1975).

Plant Nutrition

It has been demonstrated that mycorrhizae can increase plant growth. Mosse (1973) has shown that this increase is greatest in soils of low fertility. Most investigators have found that mycorrhizal plants contain a higher concentration of phosphorus than do non-mycorrhizal plants yet both types of plants utilize the same sources of soil phosphate. Zinc, copper, and sulfur are also consistently increased in mycorrhizal plants and there are some reports of calcium, magnesium, manganese, and iron increases.

Plant nutrition can affect the ability of air pollutants to cause damage. The levels of nitrogen, phosphorus, and potassium in the plant can alter the incidence and severity of air pollution injury. Ozone phytotoxicity in tomato and spinach increased with increases in supplied phosphorus (Leone and Brennan, 1970; Brewer et al., 1961) while tobacco and spinach were shown to be more sensitive to ozone with increased nitrogen nutrition (Leone et al., 1960; Brewer et al., 1961). Potassium levels also have been shown to affect oxidant injury on spinach (Brewer et al., 1961).

Although there is a great deal of literature on the effects of air pollutants on plant injury, there is virtually no information available on the influence of air pollution on soil microorganisms, particularly mycorrhizae. Grzywacz (1971) reports that sulfur dioxide and flourine decrease the frequency of association of ectomycorrhizae with tree rootlets. Sobotka (1964) found that on spruce damaged with SO_2, the ectomycorrhizal associations were abnormal, the Hartig net was hypertrophied, and the mantle was thin or absent. We have recently found that endomycorrhizal citrus exposed to ozone had mycorrhizal spore populations reduced 30% and these exposed plants showed a height decrease of 37% compared to the controls.

Conclusions

While plant responses to air pollutants are well documented, much more research is needed to determine the extent of interaction between these pollutants and microorganisms associated with the plant. Of possible importance is the secondary effect of air pollutants on endomycorrhizae. Many factors are able to decrease mycorrhizae in the soil. Among these are excess water, plant nutrition, pesti-

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About This Issue

In the spring of 1977 the University of California through its Statewide Air Pollution Research Center (SAPRC) sponsored a series of informal research workshops at UC Riverside on problems related to air pollution. Funding was provided from a special bill, AB 2189, passed by the California Legislature and signed by Governor Brown in October 1976, and from the Regents' Program to Improve Dissemination of Research Results.

The subjects and dates of the workshops and their chairmen are given below:

Non-Criteria Nitrogenous Air Pollutants, March 3-4, 1977 – Dr. James N. Pitts, Jr., Chairman; Plant, Soil Microflora, Air Pollutant Interactions, March 10, 1977 – Dr. Anton G. Endress, Chairman; Particulate Pollutants: Atmospheric Transformations and Health and Visibility Implications, April 21-22, 1977 – Dr. Daniel Grosjean, Chairman; "Air Pollutant Stressed Ecosystems: Coupling Simulation Models to Environmental Management Tasks, April 28-29, 1977 – Dr. Paul R. Miller, Chairman; and "Plant Responses to SO_2–O_3 Combination Exposures," June 2-3, 1977 – Mr. Ronald J. Oshima, Chairman.

The general goals of the workshops were a critical examination of the current state of knowledge in the particular area, an identification of research priorities, an indication of the important questions which need to be answered, and some assessment of research programs required to supply the answers. In addition to making their presentations in the programs, the speakers were invited to contribute written papers on the topics they discussed. These papers will compose four issues of Volume 7 of CALIFORNIA AIR ENVIRONMENT. Thus, it is hoped that current information in air pollution research will be more widely disseminated to those interested in air pollution problems.

The present issue, Volume 7, Number 1, contains papers from two of the workshops devoted to examination of the effects of air pollutants on plant systems, "Plant, Soil, Microflora, and Air Pollutant Interactions" and "Plant Responses to SO_2–O_3 Combination Exposures." The three papers by Mr. McCool and Dr. Menge, Dr. Manning and Dr. Tingey are from the first workshop; the paper by Dr. Bennett is from the second workshop.

CHRONIC FOLIAR OZONE INJURY: EFFECTS ON PLANT ROOT DEVELOPMENT AND POSSIBLE CONSEQUENCES

William J. Manning*

Ozone, as part of the photochemical oxidant complex, is known to be widely distributed and to adversely affect a large number of plants. Much of the injury observed is chronic or cumulative as it results from repeated exposure to low concentrations of ozone over long time periods. Expanding leaves (65-95% of full size) may exhibit extensive pigmented or white stippling, or spotting and/or chlorosis followed by premature leaf fall. The over-all effect is one of apparent retardation of growth due to early plant maturity.

Investigations of chronic ozone injury in plants has centered around determining effects on leaves, flowers, fruit, and polien. Roots and belowground stems are usually not considered, often due to the difficulty in separating intact root systems from soil for analysis. It is well-known, however, that conditions or factors which adversely affect the growth or metabolism of the leaves and shoots of plants have secondary or indirect effects on plant root growth and development due to interference with or slowing of translocation of the products of photosynthesis from leaves to roots.

Plant growth and metabolism exert a stimulatory effect on the microflora near plant roots. This zone of interaction is limited to several millimeters in width and is called the rhizosphere. The rhizosphere microflora can affect mineral nutrient availability and may influence the activities of soil-borne fungi that cause root disease.

In one of our earlier investigations, we considered the effects of chronic foliar ozone injury on root development and the rhizosphere microflora of ozone-sensitive pinto bean plants. Root systems of plants exposed to ozone were smaller in size and had poorly developed secondary roots. Many root tips were decayed and dark lesions were present on most underground stems. At the termination of the experiment, we observed that nitrogen-fixing nodules (a symbiotic association between legume roots and the bacterium *Rhizobium*) were few or absent on roots of plants exposed to ozone. The same saprophytic fungi were isolated in the same colonizing sequences from the rhizosphere and root surfaces of plants exposed and not exposed to ozone at all sampling periods. Yet the frequency of fungal isolations from roots of plants exposed to ozone was increased due to ozone-induced early plant maturity and subsequent enhanced rate of normal root breakdown which stimulates the activity of saprophytic fungi normally found on root surfaces.

Since the biological fixation of nitrogen (one of the three key elements required for plant growth) by Rhizobium nodules is an important part of the nitrogen cycle, the investigation of the effects of chronic foliar ozone injury on Rhizobium nodulation of pinto bean plants was continued. Results are summarized in Table I. Plant height and dry weight of plant tops were significantly reduced by ozone. Root dry weights and nodule numbers were considerably, but not significantly reduced. The weight and size of the nodules, however, was significantly and greatly reduced due to the secondary or indirect effects of chronic foliar ozone injury.

In a later investigation, we considered the possible effect of chronic foliar ozone injury on incidence of a root disease, i.e., the incidence of Fusarium wilt (caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *lycopersici*) in a wilt-susceptible tomato (cv. Rutgers) and in a wilt-resistant tomato (cv. Supersonic). Both are moderately ozone-sensitive. Incidence of plant death for the wilt-susceptible tomato was determined (Table II). Plants in *Fusarium*-infested soil that were exposed to ozone were smaller and slower to develop than were comparable plants in a non-ozone environment. As a result, plant deaths caused by *Fusarium* were fewer and occurred 14-18 days after they were noted in the more vigorous plants growing in the ozone-free environment. Wilt-resistant plants grown in *Fusarium*-infested soil did not develop wilt symptoms, whether exposed to ozone or not. *Fusarium* alone, however, significantly reduced root growth of the wilt-resistant tomato (Table III). Foliar ozone injury also had the same result. Ozone and *Fusarium*, singly or together in an additive manner, significantly reduced growth and root development of a *Fusarium*-resistant tomato.

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Table I. Response of Pinto Bean Plants to Repeated Exposures to a Low Concentration of Ozone

TREATMENTS	HEIGHT (cm)	DRY WTS (g) Tops	DRY WTS (g) Roots	RHIZOBIUM NODULES No.	RHIZOBIUM NODULES Wt. (g)
Ozone[a]	46.3x[b]	1.5x	0.4x	18.1x	0.239x
No Ozone	61.7y	2.9y	0.8x	29.0x	0.895y

[a] 6 pphm ozone, 5 days/week, from date of planting, 40 days duration.
[b] Each numerical value is the mean of three replications with six plants per treatment per replication. Means followed by the same letter are not statistically significantly different from each other.

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Table II. Incidence of Plant Death Due to *Fusarium* in a Wilt-Susceptible Tomato as Influenced by Repeated Exposures to a Low Concentration of Ozone

TREATMENTS	NO. DEAD PLANTS BY DAYS IN A 20-DAY PERIOD[b]					
	2	8	14	16	20	20
Ozone[a]	-	-	-	-	2	3
No Ozone	-	1	2	4	7	8

[a] 6-8 pphm ozone, 5 days/week, from date of transplanting. 45 days duration.
[b] 12 plants were used for each treatment.

Table III. Response of Wilt-Resistant Tomato Plants to *Fusarium* and Repeated Exposures to a Low Concentration of Ozone

TREATMENTS	HEIGHT (cm)	DRY WTS. (g) Tops	Roots
Ozone[a]			
Wilt-resistant + *Fusarium*	53.1w[b]	12.4w	2.5w
Wilt-resistant control	60.4x	14.8x	3.8x
No Ozone			
Wilt-resistant + *Fusarium*	61.1x	19.4y	4.8x
Wilt-resistant control	66.2y	23.9z	11.7y

[a] 6-8 pphm ozone, 5 days/week, from date of transplanting. 48 days duration.
[b] Each numberical value is the mean of five replications with three plants per treatment per replication. Means followed by the same letter are not statistically significantly different from each other.

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Results summarized here are examples of some of the experiments that we have conducted under controlled experimental conditions. They may indicate some of the possible consequences of the effects of chronic foliar ozone injury on plant root development in relation to Rhizobium symbiosis or susceptibility to root diseases. A good deal of research under actual field conditions is needed before meaningful conclusions can be drawn. Field comparsions will require the identification or development and use of closely related cultivars of plants that differ primarily in their susceptibility of ozone. Investigations should also focus on the effects of chronic foliar ozone injury on roots of perennial plants, such as trees and shrubs, and whether such injury might predispose them to soil-borne root diseases caused by weakly parasitic fungi.

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cides, deficiency or excess of root carbohydrates, deficiency in growth hormones, shading, or even pruning. Analogies can be drawn between many of these factors and the effects caused by air pollutant damage on plants. For example, reduction of the photosynthetic capacity of the leaves due to pollutant damage might have the same effect as shading the leaves. Other parallels can be made in a similar fashion.

It can be speculated that air pollution injury to the plant may reduce mycorrhizal populations which would then affect plant nutrition and lower subsequent yields. Additional information is needed to more rigorously define the extent of this interaction and the effects of air pollution on the ecological aspects of the environment, particularly the soil microenvironment.

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EFFECTS OF OZONE ON ROOT PROCESSES

David T. Tingey*

Terrestrial plants grow in two different types of environments: the shoot grows in the air and the roots grow in the soil. Plants are autotrophic but the individual organs are heterotrophic, depending upon one another for specific assimilated or elaborated products. Growth and yield are the end products of the biochemical processes of the root and shoot as related to uptake, assimilation, biosynthesis and translocation. The various plant organs extract raw materials from the air and soil and use them in biosynthetic processes to produce the required compounds for plant growth. These products are frequently produced in excess at the site of synthesis and are translocated throughout the plant. If the distribution of assimilated or elaborated substances among the plant's organs is disrupted, a reduction in growth and/or yield can result.

Ozone is a gas injurious to plant growth, entering the plant mainly through the leaves where it also exerts its primary effect. Even though the primary site of ozone action is the foliage, effects are seen in the growth, yield and quality of other plant organs. Studies with alfalfa, soybeans, tobacco and radishes show that ozone suppresses root growth more severely than foliage growth.

The observed ozone inhibition of root growth could result either directly from ozone penetrating into the soil, or indirectly by altering foliar metabolism and reducing the quality and quantity of photosynthate to the roots.

The indirect nature of the ozone inhibition of root growth was demonstrated by experiments showing that ozone diffused less than 20 mm into soil. Also, a 20 mm soil layer was effective in removing all the ozone from an air stream drawn through the soil. These results show that ozone does not penetrate the soil to any appreciable depth and could, therefore, not directly reduce root growth.

The indirect nature of ozone inhibition on root growth was confirmed by the following experiment. Plants were exposed to ozone. The foliage of half the plants was enclosed in plastic bags; the foliage of the rest of the plants was unenclosed. Root growth was reduced only in those plants in which the ozone could penetrate into the foliage. There was no significant ozone effect on the plants when ozone only reached the soil.

The root and shoot compete for photosynthate and other resources needed for their growth and development. When shoot-synthesized carbohydrates become too deficient to maintain the normal growth rate, sinks such as roots and fruits are more severely affected than the shoot. This is consistent with the observation that the function of organs remote from the site of synthesis or absoption are more severely limited under stress conditions. Hence, when either storage roots, fruits or seeds have a high demand for photosynthate, they will experience a reduction in translocated photosynthate and growth will be reduced if an ozone stress has reduced photosynthesis during this period. This principle suggests that ozone causes a depletion or reduction in carbohydrate levels in roots. Studies with alfalfa and pine exposed to chronic ozone levels showed a reduction in total photosynthate in the plant, a retention of carbohydrates in the foliage, and a concurrent reduction in the roots. Similar alterations in foliage and root levels of amino acids, proteins and phenols also occur as a result of ozone exposure.

When root carbohydrates are deficient the roots do not have the energy necessary to drive their matabolic processes. Mineral uptake in plants exposed to ozone is reduced as would be predicted by a lower root carbohydrate level. Also, symbiotic nitrogen fixation, the conversion of atmospheric nitrogen to organic nitrogen by leguminous plants, is severely inhibited by ozone. Nitrogen fixation is, in part, controlled by amount of photosynthate in the roots.

The above data show that ozone alters root growth and root processes by first reducing photosynthesis and altering foliar metabolic pathways. The alteration in foliar metabolism is reflected in lowered carbohydrate levels in the roots. This can reduce key metabolic processes such as mineral uptake and symbiotic nitrogen fixation. These changes in root metabolism will ultimately be expressed in reduced plant growth and yield.

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EXPERIMENTAL DESIGN CONSIDERATIONS FOR MIXED GAS STUDIES

James P. Bennett*

Studies of the effects of mixtures of air pollutants on plants should be designed in such a way that they demonstrate responses which are different from those produced by the gases used alone. Developing a proper design is an endeavor suitable for statisticians who are working in the field of experimental design. The problem of detecting a unique mean mixture effect is not new to the field of statistics and designs already exist that can help the air pollution scientist. Two basic approaches to these designs will be described in this article: the analysis of variance (AOV) design and the regression (REG) design. In the former, one is concerned with determining the probability of a given mean effect occurring and in the latter, how much of the mean mixture effect actually exists.

What we are looking for is how two gases act together in causing a plant response in contrast to acting alone. In statistical terms this is called *interaction* and is detected by allowing both factors to vary. In a classical two-way AOV design, several levels of each factor are allowed to vary in all possible combinations, e.g., if there are three levels of two factors, a total of nine possible treatments can occur and need to be used. In the Type 2 design (Figure 1), the brown solid line surrounds the four separate treatments of two levels of O_3 and SO_2 used alone. The control treatment (no pollutants) has been left out of the Type 2 illustration. The brown dashed line surrounds the four possible combinations of the two pollutants in mixtures. The analysis of variance table below shows how there are two degrees of freedom (DF) for each single gas treatment, and four degrees of freedom for the interaction between the two gases in mixture.

The conventional mixed gas study has been set up like the Type 1 design in Figure 1. There is only one level of each gas alone and a control, giving two degrees of freedom for single gases. There is one combination treatment which does not vary concentration so there is no degree of freedom for variability to occur. Consequently there is no possible way for an interaction to occur. In the Type 2 design (Figure 1) the blue solid lines separate out *two* designs identical to the Type 1, leaving the obvious interaction mixtures, $[O_3]_2 + [SO_2]_1$ and

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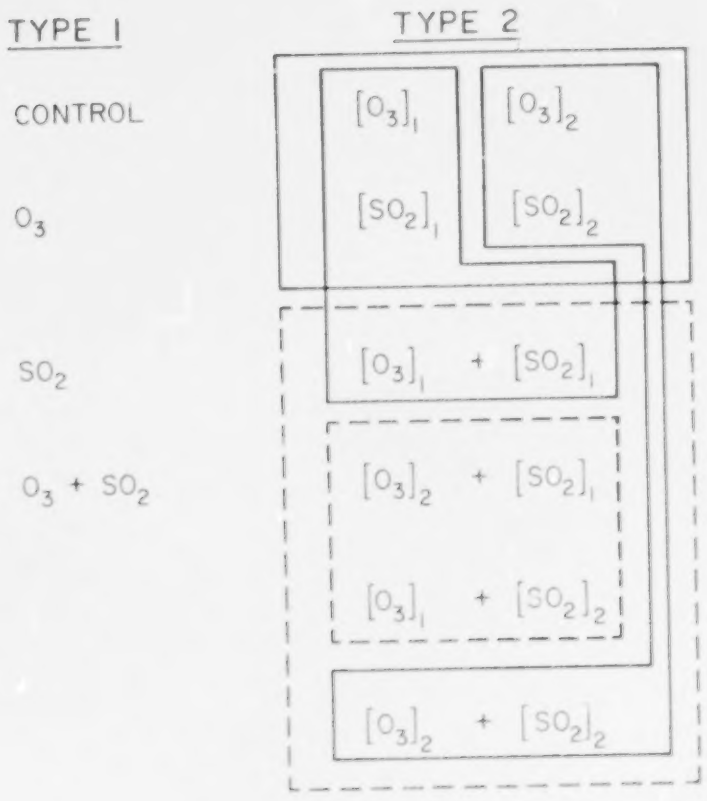

ANALYSES OF VARIANCE

	TREATMENT	DF	TREATMENT	DF
(1)	C, O_3, SO_2	2	O_3	2
(2)	$O_3 + SO_2$	0	SO_2	2
	(1) x (2)	0	O_3 x SO_2	4

Figure 1. Interaction designs for air pollution studies (O_3 – Ozone; SO_2 – Sulfur Dioxide; C – Control; DF – Degress of Freedom. Subscripts 1 and 2 outside the brackets [ ] indicate different concentrations).

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$[O_3]_1 + [SO_2]_2$ (blue dashed line) out of the study.

The interaction term in the analysis of variance will partition out the portion of the total variance which is attributable to the two gases varying together. Replication within cells is strictly necessary so as to provide an error term in the AOV. When the interaction variance is compared to the residual variance in an F test one will be able to decide whether or not the interaction was large enough to be significant. If this is decided then the effect of each gas alone means very little since the effect of one depends on the other, and vice versa. The observed F value can be looked up in a table of expected F values to determine the probability of getting the observed F and thus the probability of the interaction variance being larger than the residual variance.

We have thus far determined the probability of the interaction occurring. How do we now quantify it so we can tell how much of an interaction occurs? This can be studied by means of regression analysis. Table I summarizes the main differences between the two types of approaches.

In a regression design, one would set up many fumigations of many different levels of each gas alone and in combination. For example, one might use ten levels of O_3 and ten of SO_2 plus ten levels of O_3 + SO_2. Replication is desirable but not strictly necessary. Regression analysis then calculates how much the response parameter (e.g., dry weight) varies with the air pollutant concentration or dose (the independent variable), called covariance, and this is compared to the variance in the concentration itself. This calculation produces a parameter called the slope, which tells how much the response varies with the concentration, e.g., grams per ppm. This is a quantitative estimate of how much dry weight decreases with concentration and would be written

$$dry\ weight = a - b\ (concentration)$$

where a is the intercept of the line and b is the slope (Figure 2). Some response functions may be more complex than a straight line and a statistician should be consulted for assistance at that point.

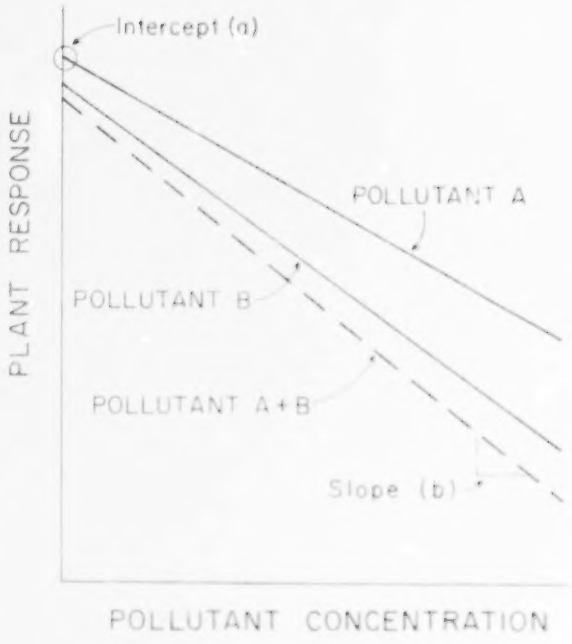

Figure 2. Schematic presentation of interaction results using a regression design. Lines would be plotted through data points using many concentration treatments. The slope of Pollutant A + B could occur below or between Pollutant A and Pollutant B.

A regression line can be calculated for each gas alone and the mixture giving three slopes (Figure 2). It is not possible to calculate four slopes, i.e., one for each gas alone and in the presence of the other gas because the design does not hold one gas concentration fixed while the other varies. The combination fumigation must include high levels of one gas with low levels of the other, and vice versa. For each mixed concentration there are two possible combinations: e.g., high O_3 with low SO_2 and low O_3 with high SO_2. The slope of this mixed gas regression line can then be compared statistically with the other two slopes. If a significantly different slope is found, one can conclude an interaction exists between the two gases.

Transformations are sometimes necessary to normalize data for regression analysis. The most common one in dose-response studies is the probit transformation but this is restricted to discrete qualitative variables that are binomially distributed, e.g., mortality in test mice. In plant effects studies, we are usually dealing with continuous variables that are normally distributed, e.g., dry weights. For this kind of data any transformation that linearizes the regression function is suitable for dose-response regression analysis. A large body of literature from the pharmacological sciences exists on the use of probit analysis which defines algebraically and statistically what an interaction is but this should be used with caution.

Anyone embarking on a mixed gas fumigation study would do well to seek the advice and aid of a statistician trained in the field of experimental design. A proper analysis is not possible without a well-designed experiment.

Table I. Comparison of Analysis of Variance and Regression

ANALYSIS OF VARIANCE	REGRESSION
A few levels of a factor	Many levels of a factor
Significance tests on means	Significance tests on slopes
No estimate of cause and effect	Cause and effect estimates
Effects not quantifiable	Effects are quantifiable
Qualitative variables	Quantitative variables
Replication needed	Replication not strictly necessary

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